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BIOSYNTHESIS OF *ANDROGRAPHIS PANICULATA* REDUCED SILVER-MAGNESIUM OXIDE MICRO-COMPOSITE AND IT'S ANTIMICROBIAL ACTIVITY.

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ABSTRACT

The aim of this study to prepare Ag-MgO core-shell micro-composites of *Andrographis Paniculata* extract and assess its effectiveness against *Escherichia coli*. Micro-composite was synthesized by magnesium oxide was coated on silver microparticles of *Andrographis Paniculata* extract under sonication technique. The phytochemical analysis of *Andrographis Paniculata* extract was done in water (polar), acetone (dipolar) and n-hexane (non-polar) solvents and showed the presence or absence of phenolic compounds, saponin, alkaloids, tannins, flavonoids and steroids. Synthesized micro-composites were examined by a UV-Visible spectrophotometer, quantitative calibration curve analysis at 297 nm, FTIR spectroscopy and scanning electron microscopy. The prepared Ag-MgO micro-composites were also examined against *Escherichia coli* for determining its efficiency. The prepared Ag-MgO micro-composite showed distinctive bands at 297 nm for Ag-MgO micro-particle. The prepared micro-composite shown higher antibacterial activity against *Escherichia coli*, indicating a higher interaction between Ag and MgO ions. In nutshell, Ag-MgO micro-composites of *Andrographis Paniculata* extract was successfully prepared and shows significant antimicrobial activity.

INTRODUCTION

Waterborne epidemics are related to high faecal contaminants like pathogenic microbes, which are a worldwide problem. The treatment of infectious water related disease causes high resistance to causative microbial agents, resulting in an increasing number of deaths across the globe, mostly in the children.[1] Now, researchers are exploring the eco-friendly material toward the development of effective Anti-bacterial nano material against multidrug-resistant human pathogens and one of them is *Escherichia coli*. It is the life threatening contaminant in drinking water. Non-toxic and eco-friendly plant derived extract are reliable biosynthesis agents for metal oxide nanoparticles synthesis.[2, 3] The *Andrographis Paniculata* a member of *acanthaceae* family has been showed the presence of higher contents of phytochemicals, resulting showed high antimicrobial activity.[4] Among the metallic and metal oxide nanoparticles, silver

(Ag) and magnesium oxide (MgO) nanoparticles have showed broad-spectrum antibacterial activity. In recent studies, it showed that nanocomposite produces stronger antibacterial activity against *E.coli*.[5, 6]

MATERIALS AND METHODS

Plant Collection: Fresh, mature leaves of *Andrographis Paniculata* leaves were collected from botanical garden of HSBPVT'S, GOI, College of Pharmacy, Kashti, Shrigonda, Ahmednagar, Maharashtra, India. Silver nitrate was obtained as a gift sample by Ultraquery Technologies India Private Limited, Pune. Magnesium nitrate hexahydrate was purchased from Advent Chembio Private Limited, Navi Mumbai and sodium hydroxide was purchased from Annexe Chem Private Limited, Vadodara.

Preparation of *Andrographis Paniculata* Extract: The collected plant leaves were washed twice in sterile distilled water to eliminate earthy material like soil and salts. The washed leaves of plant were shade dried for 10 days at room temperature. The dried leaves were subjected to grind for formation of powder. Then 10 gm of plant powder was added into 100 ml of distilled water and boiled for 5 min. The boiled extract was filtered through Whatman No. 1 filter paper and then supernatant was used for further process.

Synthesis of AgMP's: For the synthesis of *Andrographis Paniculata* reduced silver microparticles, add 10 ml of pure plant extract into 90 ml of 1 mM of silver nitrate in 250 ml of conical flask. This mixture was kept at room temperature under mechanically stirring. The change in color showed formation of *Andrographis Paniculata* reduced silver particles solution. Then this solution was subjected toward sonication process.[7]

Synthesis of Ag-MgO Micro-composite: The Ag-MgO micro-composite were synthesized by seed growth process going through bioreduction by the precipitation method at room temperature using the *Andrographis Paniculata* leaves extract as natural reducing agent in the presence of metallic precursors. In synthesis process, first add 80 ml of prepared *Andrographis Paniculata* reduced silver microparticles solution with 20 ml of 0.1 M $Mg(NO_3)_2.6H_2O$ under continuous stirring for 2 hours at room temperature. Then subsequently 2 ml of 0.1 M NaOH solution was added dropwise until a complete precipitation. Then Ag-MgO solution was subjected to sonication technique. The product obtained was centrifuged (4500 rpm) for 30 min. filtered and freeze dried.[8]

Phytochemical analysis:

Sample collection[9]

Extraction Method and Isolation Technique

Maceration: In this extraction method, coarsely powdered *Andrographis Paniculata* leaves powder was kept in contact with the each solvent in airtight containers for 7 days with frequent agitation, as result soluble matters were dissolved and filter through Whatman No. 1 filter paper and collect test solution.[10]

Testing methods-

Phenolic compound: Acetone, n-hexane and water extract were separately treated with one drop of ferric chloride. Yellow colour formation shows the presence of phenolic compounds.

Saponin: The test solution of each solvents were separately shaken well with water. Foamy layer formation shows the presence of saponin.

Alkaloids: The extract of acetone, n-hexane and water were separately treated with 2N HCl and filtered. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate shows the presence of alkaloids.

Tannins (Gelatin test): The extracts were separately treated with 1% gelatin solution containing sodium chloride. White precipitate formation shows the presence of tannins.

Flavonoids: Extracts were separately treated with few drops of lead acetate solution. Yellow colored formation shows the presence of flavonoids.[11]

Table 1: Plant Extraction in Different Solvents.

Polar	Dipolar	Non-polar
Water	Acetone	N-hexane

Table 2: Phytochemical Analysis in Different Solvents.

Phytochemical analysis	Water	Acetone	N-hexane
Phenolic compound	+	+	+
Saponin	+	+	+
Alkaloid	+	+	-
Tannin	+	+	+
Flavonoid	+	-	-
Steroid	+	+	+

Where; (+): Presence and (-): Absence.

From result, the phytochemical analysis of *Andrographis Paniculata* shows maximum solubility of phytochemicals in water as compared to acetone and n-hexane.[12]

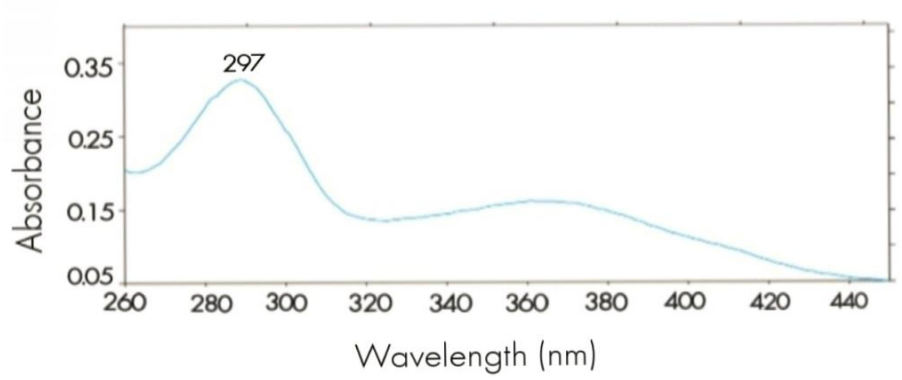

Figure 1: UV-Visible analysis of Ag-MgO micro-composite

UV-Visible analysis

Fig. 1 shows UV-Visible absorbance spectra of Ag-MgO micro-composite, as shown in spectrum peak was observed in the low-UV region at 297 nm wavelength with 0.961192 AU absorbance.[13]

Table 3: Quantitative Calibration Study at Different Concentrations.

Concentration (ppm)	**Absorbance (AU)**	**297 nm**
2	0.0192786	0.0193
4	0.0395741	0.0396
6	0.0540462	0.0540
8	0.0752045	0.0752
10	0.0928910	0.0963

Functional group	**Peak observed**
3222	OH
1594	C=C
1500	N-O
1394	OH (Phenol)
1232	C-O-C (Lactone ring)
600	Mg-O-Mg

Table 4: Presence of Functional Groups in FTIR region.

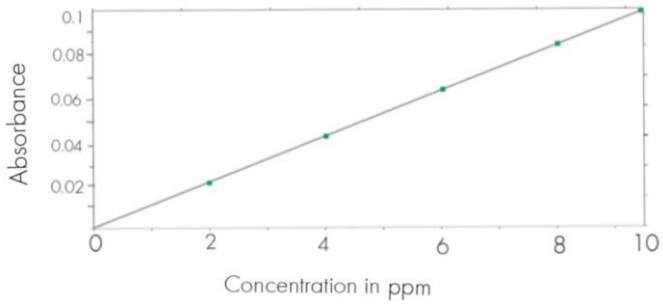

Figure 2: Calibration curve of Ag-MgO micro-composite at 297 nm.

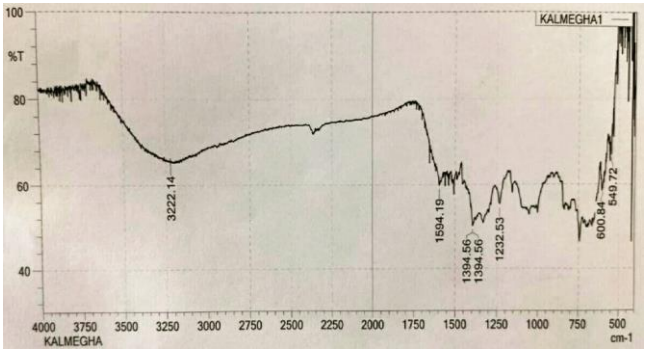

Figure 3: FTIR Spectroscopy of Ag-MgO micro-composite

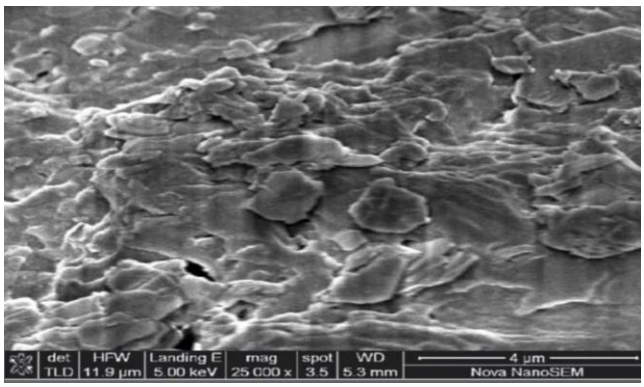

Figure 4: SEM Image of Ag-MgO micro-composite

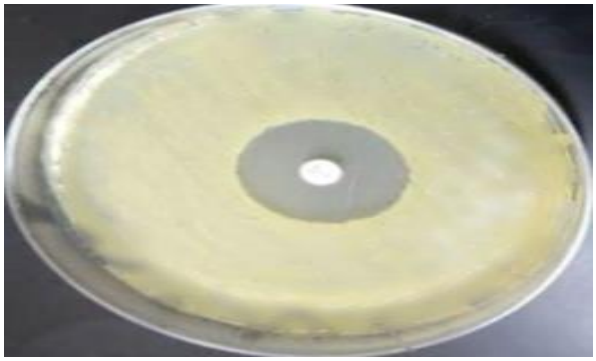

Figure 5: Well diffusion Assay of Ag-MgO micro-composite against *E.coli.*

Steroids: Extracts were separately treated with 3 drops of acetic anhydride and 2 drops of conc. H_2SO_4. Green colored formation indicates the presence of steroids.[12]

Physicochemical and mineralogical characterization of Ag-MgO micro-composite

The synthesized *Andrographis Paniculata* leaves extract reduced Ag-MgO micro-composite solution absorption range estimation and quantitative calibration study were done by using UV- Visible Spectrophotomer (LMSP-UV1900). All possible functional groups, which are present in *Andrographis Paniculata* leaves extract reduced Ag-MgO micro-composite was studied by FTIR Spectroscopy. The FTIR was recorded in the range of 500-4000 cm^{-1}. The surface morphology of the Ag-MgO micro-composite was studied on SEM (FEI Nova Nano SEM 230).

Antibacterial evaluation of the Ag-MgO micro-composite: Bacterial resistance of micro-composite was determined by observed zone of inhibition on standard agar well disc diffusion method (Kirby-Bauer disk diffusion test). *Escherichia coli* strain was used as indicator. Bacterial suspension were prepared. 60 µL of Ag-MgO solution was added over cellulose nitrate sterile filter paper with pore size of 0.45 µm and allowed to dry at room temperature for 4 hours and placed on MacConkey nutrient agar plate, which is already inoculated by bacterial cell suspension. The plate was incubated at 37°C for 24 hours and zone of inhibition was measured in diameter (mm). Minimum inhibitory concentration of biosynthesized Ag-MgO micro-composite was determined by using the microtiter broth-dilution method.[8]

Quantitative calibration study: Determination of linearity was done by using five concentrations of standard solution of Ag-MgO micro-composite in methanol solvent. As the concentration (part per million) increase, absorbance also was increased at 297 nm. As showed in fig. 2, all points are passed through straight line curve of absorbance vs. concentration graph plot with correlation coefficient of 0.99813 and standard error of 0.193704. As result, this proved that, given formulation follows Beer's-Lambert law.

FTIR analysis: As shown in fig. 3, the presence of functional group of the various metabolites in *Andrographis Paniculata* leaves extract are responsible for the bioreduction, capping and stabilizing of the Ag-MgO micro-composite were identified between 4000 to 500 cm^{-1} of FTIR region.[14, 15]

Antibacterial activity study

Well diffusion assay method: In agar well diffusion, experiment was carried out on Ag-MgO micro-composite using *Andrographis Paniculata* leaves extract. In result, it was showed inhibition zone of 24 mm in diameter against *Escherichia coli.*

MIC Method: In MIC, experiment was carried out on *Andrographis Paniculata* leaves extract reduced Ag-MgO micro-composite. In that, formulation does record 25 µg/ml concentration as minimum inhibitory concentration against *Escherichia coli*.[17, 18, 19]

CONCLUSION

The phytochemical analysis of *Andrographis Paniculata* leaves extract was performed. Synthesis of Ag-MgO micro-composite by using *Andrographis Paniculata* leaves extract was done. Evaluation of Ag-MgO micro-composite using *Andrographis Paniculata* leaves extract were done by UV-Visible analysis, quantitative calibration curve study, FTIR spectroscopy and SEM analysis. Also antibacterial activity and reduced dose concentration were confirmed by well diffusion assay and MIC method respectively against *E.coli*.

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Conflict of interest: The authors declare no conflict of interest.

Abbreviations used: Ag-MgO: magnesium oxide coated silver microparticle, AgMP's: silver microparticles, Mg $(NO_3)_2.6H_2O$: magnesium nitrate hexahydrate, AU: Absorbance unit, FTIR: Fourier transform infrared radiation, SEM: Scanning electron microscopy, MIC: Minimum inhibitory concentration

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